Surveillance of Wildlife Diseases from the National Forestry and Grassland Administration.

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References

1. Gu M, Liu W, Cao Y, Peng D, Wang X, Wan H, et al. Novel reassortant highly pathogenic avian influenza (H5N5) viruses in domestic ducks, China. Emerg Infect Dis. 2011;17:1060–3. https://doi.org/10.3201/eid/1706.101406
2. Wu H, Peng X, Xu L, Jin C, Cheng L, Lu X, et al. Novel reassortant influenza A(H5N8) viruses in domestic ducks, eastern China. Emerg Infect Dis. 2014;20:1315–8. https://doi.org/10.3201/eid2008.140339
3. Cui Y, Li Y, Li M, Zhao L, Wang D, Tian J, et al. Evolution and extensive reassortment of H5 influenza viruses isolated from wild birds in China over the past decade. Emerg Microbes Infect. 2020;9:1793–803. https://doi.org/10.1080/22221751.2020.1797542
4. Global Consortium for H5N8 and Related Influenza Viruses. Role for migratory wild birds in the global spread of avian influenza H5N8. Science. 2016;354:213–7. https://doi.org/10.1126/science.aaf8852
5. Li M, Liu H, Bi Y, Sun J, Wong G, Liu D, et al. Highly pathogenic avian influenza A(H5N8) virus in wild migratory birds, Qinghai Lake, China. Emerg Infect Dis. 2017;23:637–41. https://doi.org/10.3201/eid2304.161866
6. Li Y, Li M, Li Y, Tian J, Bai X, Yang C, et al. Outbreaks of highly pathogenic avian influenza (H5N6) virus subclade 2.3.4.4h in swans, Xinjiang, western China, 2020. Emerg Infect Dis. 2020;26:2956–60. https://doi.org/10.3201/eid2612.201201
7. Lewis NS, Banyard AC, Whittard E, Karibayev T, Al Kafagi T, Chvala I, et al. Emergence and spread of novel H5N8, H5N5 and H5N1 clade 2.3.4.4 highly pathogenic avian influenza in 2020. Emerg Microbes Infect. 2021;10:148–51. https://doi.org/10.1080/22221751.2021.1872355
8. Saito T, Tanikawa T, Uchida Y, Takemae N, Kanehira K, Tsunekuni R. Intracontinental and intercontinental dissemination of Asian H5 highly pathogenic avian influenza virus (clade 2.3.4.4) in the winter of 2014–2015. Rev Med Virol. 2015;25:388–405. https://doi.org/10.1002/rmv.1857
9. Sorensen MC, Dixit T, Kardynal KJ, Newton J, Hobson KA, Bensch S, et al. Migration distance does not predict blood parasitism in a migratory songbird. Ecol Evol. 2019;9:8294–304. https://doi.org/10.1002/ece3.5404
10. Li S, Meng W, Liu D, Yang Q, Chen L, Dai Q, et al. Migratory whooper swans *Cygnus cygnus* transmit H5N1 virus between China and Mongolia: combination evidence from satellite tracking and phylogenetics analysis. Sci Rep. 2018;8:7049. https://doi.org/10.1038/s41598-018-25291-1

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Rapid Antigen Test for Postmortem Evaluation of SARS-CoV-2 Carriage

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Detecting severe acute respiratory syndrome coronavirus 2 in deceased patients is key when considering appropriate safety measures to prevent infection during postmortem examinations. A prospective cohort study comparing a rapid antigen test with quantitative reverse transcription PCR showed the rapid test's usability as a tool to guide autopsy practice.

Rapid detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is essential to prevent viral dissemination. Rapid antigen tests (RATs) have recently been approved and are now widely used in the current coronavirus disease (COVID-19) pandemic (1). Although the performance of RATs has been evaluated extensively in clinics (2–4), data on postmortem testing are still lacking (5).

We performed a prospective cohort study in which we evaluated the performance of the Roche/SD Biosensor SARS-CoV-2 RAT (https://www.roche.com) in 30 consecutive deceased COVID-19 patients at the University Hospital, Medical University of Graz (Graz, Austria), during November 28–December 23, 2020. We tested each corpse with nasopharyngeal swabs for RAT (using the manufacturer's kit) and eSwabs (https://www.copanusa.com) for quantitative reverse transcription PCR (qRT-PCR) targeted to the viral envelope (E) and nucleocapsid (N) genes of SARS-CoV-2. Furthermore, we used virus isolation from lung tissue swabs from an additional cohort of deceased COVID-19 patients (n = 11) to compare molecular detection and virus cultivability (Appendix, https://wwwnc.cdc.gov/EID/article/27/6/21-0226-App1.pdf).

All patients were Caucasian, median age was 78 years (range 62–93 years), and 51.2% were female. The median disease duration (interval between the first positive SARS-CoV-2 PCR and death) was 11 days (range 1–43 days). The median postmortem interval (time between death and specimen sampling) was 23 hours (range 8–124 hours; Table; Appendix).

PCR is the current standard for SARS-CoV-2 detection (*1*,*2*). In our cohort, qRT-PCR targeted to the E gene showed a higher sensitivity than qRT-PCR for

Table. Patient characteristics and postmortem data for investigation of rapid antigen test for postmortem evaluation of SARS-CoV-2 carriage, Graz, Austria*

Characteristic	RAT cohort, n = 30	Culture cohort, n = 11
Age, y, median (range)	78 (62–93)	79 (65–93)
Sex, no. (%)		
M	14 (47.7)	6 (56)
F	16 (53.3)	5 (45.4)
Disease duration,† d, median (range)	12 (1–43)	9 (3–34)
Postmortem interval‡, h, median (range)	22 (8–124)	25 (14–68)
qRT-PCR positive, no. (%)	24 (80)	11 (100)
C_t value, median (range)		
E gene	22.8 (14.1–37.3)	19.9 (13.7–36.0)
N gene	26.9 (18.0–34.6)	24.6 (17.3–33.7)
Cultivation positive, no. (%)	NA	7 (63.6)
RAT positive, no. (%)	17 (56.7%)	NA
Total RAT specificity (95% CI§), n = 30	100% (61%–100%)	NA
RAT sensitivity (95% CI§), n = 30	70.8% (50.8%–85.1%)	NA
Total, n = 30		
C_t ≤35,¶ n = 23	73.9% (53.5%–87.5%)	NA
C_t ≤30,¶ n = 18	94.4% (74.2%–99.7%)	NA
C_t ≤25,¶ n = 16	100% (80.6%–100%)	NA

*C_t, cycle threshold; E, envelope; N, nucleocapsid; NA, not applicable; qRT-PCR, quantitative reverse transcription PCR: RAT, rapid antigen test; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
†Interval from first positive (antemortem) SARS-CoV-2 PCR to death.
‡Interval from death to specimen sampling.
§Determined via the hybrid Wilson/Brown method (*10*).
¶Determined via E gene qRT-PCR.

the N gene (Appendix Figure 1). Consequently, we used E gene qRT-PCR as the reference in subsequent evaluations. Results showed that 80% (24/30) of cases were qRT-PCR positive, whereas 56.7% (17/30) were RAT positive (Figure, panel A). RAT had an overall specificity of 100% (95% CI 61%–100%) and an overall sensitivity of 70.8% (95% CI 50.8%–85.1%) when using E gene qRT-PCR as the reference. RAT negative cases showed significantly higher C_t values in qRT-PCR compared with RAT positive cases (mean 38.24 [SD 7.01] vs 20.74 [SD 3.46]; Figure, panel B). Correspondingly, RAT sensitivity increased when cases were stratified according to C_t values (C_t ≤35, sensitivity 73.9% [95% CI 53.5%–87.5%]; C_t ≤30, sensitivity 94.4% [95% CI 74.2%–99.7%]; C_t ≤25, sensitivity 100% [95% CI 80.6%–100%]; (Table; Appendix Table 1). Furthermore, when we compared qRT-PCR results from nasopharyngeal swabs of patients in which viral culture was performed (from corresponding lung tissue swabs of an additional cohort), cultivability was restricted to cases with C_t values ≤23.7, which is below the threshold of false-negative RAT cases (C_t values ≥25.8; Figure, panels B, C). These results are in line with most clinical RAT studies that also used virus culture (*2*–*4*,*6*), in which cultivability is exceedingly rare in cases with low viral loads determined with qRT-PCR. We used cultivation from lung tissue swab specimens for this analysis because the lung often shows increased SARS-CoV-2 loads in deceased patients (*7*; Appendix Table 2) and therefore represents a major infection source during autopsy.

Furthermore, we determined parameters that influenced test performance. We noted a significant positive correlation between disease duration and C_t values (Figure, panel D). Such correlation was also evident in RATs; all cases with disease courses >17 days were RAT negative (Figure, panel E). Postmortem intervals did not correlate with C_t values or RAT results (Figure, panels G, H). Thus, a long disease duration rather than a long postmortem interval seems to be the main factor for increased C_t values and negative RATs. RAT and cultivation results closely mirrored each other with respect to viral load (Figure, panels B, C), disease duration (Figure, panels E, F), and postmortem interval (Figure, panels H, I).

Although RAT had an overall lower sensitivity than qRT-PCR in this study, our data suggest that viral loads of false-negative RAT cases are probably below the threshold of cultivability. Because culture is regarded as a measure of virus viability and infectivity (*8*), these cases likely pose only minimal risks of SARS-CoV-2 transmission during postmortem examinations. However, each corpse having a postmortem evaluation must be treated as potentially infectious. Even a PCR-negative nasopharyngeal swab specimen does not exclude the presence of viable virus in other body sites, as shown in COVID-19 (*7*), thus emphasizing the general application of appropriate autopsy safety measures.

In conclusion, RAT should not be seen as a potential replacement for but rather as an addition to of current postmortem testing strategies. Especially

when qRT-PCR is not readily available, RAT might be useful in selecting the most hazardous corpses that should be examined under special conditions (e.g., Biosafety Level 3 [9]). RAT could therefore be a valuable adjunct tool in guiding autopsy practice.

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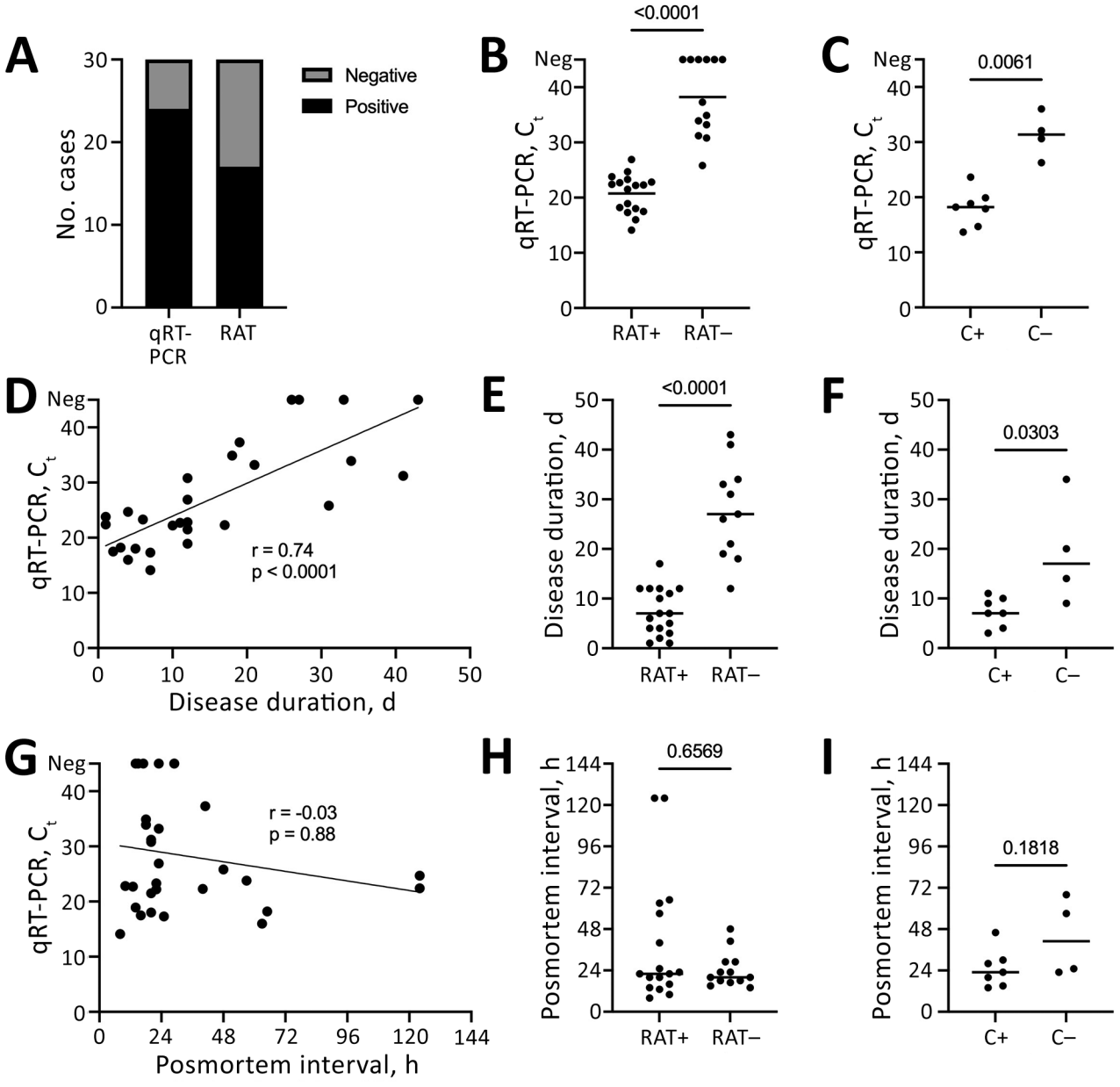

Figure. Postmortem detection and cultivation of SARS-CoV-2 for investigation of RAT for postmortem evaluation of SARS-CoV-2 carriage, Graz, Austria. A) Among 30 deceased SARS-CoV-2 patients, RAT detected fewer positive cases than did qRT-PCR. B) RAT-negative cases show significantly higher C_t values in qRT-PCR compared with RAT-positive cases (Mann-Whitney test). C) Cultivation negative and positive cases mirror C_t values of RAT results (Mann-Whitney test). D–F) Longer disease durations are significantly correlated with higher C_t values (Spearman correlation test; D), negative RAT results (Mann-Whitney test; E), and negative cultivation results (Mann-Whitney test; F). G–I) No significant correlation was found between postmortem intervals and C_t values (Spearman correlation test; G), RAT results (Mann-Whitney test; H), or cultivation results (Mann-Whitney test; I). C, cultivation; C_t, cycle threshold; neg, negative; qRT-PCR, quantitative reverse transcription PCR; RAT, rapid antigen test; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; +, positive; –, negative.

References

1. Centers for Disease Control and Prevention. Interim guidance for antigen testing for SARS-CoV-2 [cited 2021 Mar 27]. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html
2. Dinnes J, Deeks JJ, Berhane S, Taylor M, Adriano A, Davenport C, et al.; Cochrane COVID-19 Diagnostic Test Accuracy Group. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 2021;3:CD013705.
3. Albert E, Torres I, Bueno F, Huntley D, Molla E, Fernández-Fuentes MÁ, et al. Field evaluation of a rapid antigen test (Panbio™ COVID-19 Ag Rapid Test Device) for COVID-19 diagnosis in primary healthcare centres. Clin Microbiol Infect. 2021;27:472.e7–10. https://doi.org/10.1016/j.cmi.2020.11.004
4. Iglòi Z, Velzing J, van Beek J, van de Vijver D, Aron G, Ensing R, et al. Clinical evaluation of Roche SD Biosensor rapid antigen test for SARS-CoV-2 in municipal health service testing site, the Netherlands. Emerg Infect Dis. 2021 Mar 16 [Epub ahead of print]. https://doi.org/10.3201/eid2705.204688
5. Centers for Disease Control and Prevention. Collection and submission of postmortem specimens from deceased persons with confirmed or suspected COVID-19: postmortem guidance [cited 2021 Mar 27]. https://www.cdc.gov/coronavirus/2019-ncov/hcp/guidance-postmortem-specimens.html
6. Singanayagam A, Patel M, Charlett A, Lopez Bernal J, Saliba V, Ellis J, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Euro Surveill. 2020;25:2001483. https://doi.org/10.2807/1560-7917.ES.2020.25.32.2001483
7. Puelles VG, Lütgehetmann M, Lindenmeyer MT, Sperhake JP, Wong MN, Allweiss L, et al. Multiorgan and renal tropism of SARS-CoV-2. N Engl J Med. 2020;383:590–2. https://doi.org/10.1056/NEJMc2011400
8. Jefferson T, Spencer EA, Brassey J, Heneghan C. Viral cultures for COVID-19 infectious potential assessment—a systematic review. Clin Infect Dis. 2020 Dec 20 [Epub ahead of print]. https://doi.org/10.1093/cid/ciaa1764
9. Loibner M, Langner C, Regitnig P, Gorkiewicz G, Zatloukal K. Biosafety requirements for autopsies of patients with COVID-19: example of a BSL-3 autopsy facility designed for highly pathogenic agents. Pathobiology. 2021;88:37–45. https://doi.org/10.1159/000513438
10. Brown LD, Cai TT, DasGupta A. Interval estimation for a binomial proportion. Stat Sci. 2001;16:101–33. https://doi.org/10.1214/ss/1009213286

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Respiratory Viral Shedding in Healthcare Workers Reinfected with SARS-CoV-2, Brazil, 2020

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We documented 4 cases of severe acute respiratory syndrome coronavirus 2 reinfection by non–variant of concern strains among healthcare workers in Campinas, Brazil. We isolated infectious particles from nasopharyngeal secretions during both infection episodes. Improved and continued protection measures are necessary to mitigate the risk for reinfection among healthcare workers.

Coronavirus disease (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, China,

[1]These authors contributed equally to this article.

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Rapid Antigen Test for Postmortem Evaluation of SARS-CoV-2 Carriage

Appendix

Materials and Methods

Case Description

Clinical parameters were obtained from electronic medical records and are shown in Table 1 and Appendix Tables 1 and 2. The study was approved by the institutional review board of the Medical University of Graz, Austria (32–362ex19/20).

SARS-CoV-2 Quantitative Reverse Transcription PCR (qRT-PCR)

We extracted RNA from 200 μL eSwab solution using the Maxwell simplyRNA Blood Kit (Promega, https://www.promega.com) eluting RNA in 50 μL distilled water. qRT-PCR detected regions of the viral envelope (E) and nucleocapsid (N) specific to SARS-CoV-2 (*1*). Primers, probes, and 5 μL of RNA solution were added to 10 μL of SuperScript III One-Step RT-PCR System withμ Platinum *Taq* High Fidelity DNA Polymerase (Thermo Fisher, https://www.thermofisher.com) master mix. PCR was performed on a Quantstudio 7 instrument (Thermo Fisher) with the following cycling conditions: 55°C 15 min, 95°C 3 min; 45 cycles (95°C 15 sec; 58°C 30 sec). We used human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA as internal RNA control with the same cycling conditions. All primers and probes were from Eurofins Scientific (https://www.eurofins.com).

We downloaded and processed amplification data using the qpcR package of the R project (https://www.r-project.org). Amplification efficiency plots were visually inspected, and Cp2D (cycle peak of second derivative) values were calculated for samples with valid amplification curves. We generated plots with R using the reshape, tidyverse, and ggplot packages.

SARS-CoV-2 Cultivation

For SARS-CoV-2 cultivation, we used swabs from lung parenchyma collected during autopsy. Samples were frozen (−80°C) and thawed (37°C) twice to increase cell lysis and viral release. We added 2 mL OptiPro SFM medium (GIBCO, https://www.thermofisher.com) with 4 mM L-glutamine (GIBCO) and 1% penicillin–streptomycin (10,000 U/mL; GIBCO) were added to the samples. After centrifugation (10 min, 1,500 rcf) the supernatants were filtered through a 0.45 μm membrane filter (Millipore, https://www.sigmaaldrich.com) and inoculated on Vero-E6 cells with OptiPro SFM medium with 4 mM L-glutamine and 1% penicillin–streptomycin in T25 flasks (Thermo Fisher). After 3–4 days incubation at 37°C and 5% CO_2, the whole cells were detached and passaged, including the supernatant, to new Vero-E6 cells growing in T75 flasks (Thermo Fisher). After 1 week, we harvested the cells and stored the supernatants after centrifugation (10 min, 1,500 rcf) at −80°C.

Reference

1. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25:2000045. PubMed https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

Appendix Table 1. Case characteristics and postmortem data of the RAT cohort*

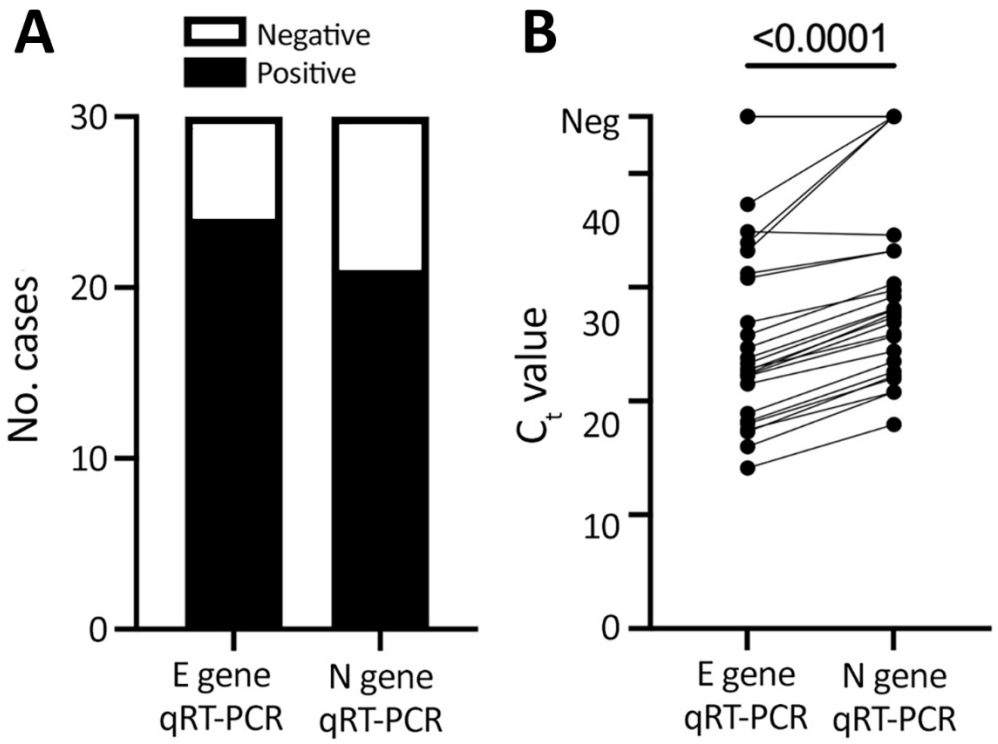
Case no.	Age, y/sex	Disease duration, d†	Postmortem interval, h‡	RAT	qRT-PCR	C_t value E gene	C_t value N gene	C_t value GAPDH
1	79/F	5	20	Positive	Positive	18	22	21.3
2	75/F	31	48	Negative	Positive	25.8	30.3	25.9
3	72/F	1	124	Positive	Positive	22.4	27.7	26.4
4	71/F	18	18	Negative	Positive	34.9	34.6	22.9
5	89/F	12	20	Negative	Positive	30.8	33.2	19.8
6	73/F	6	22	Positive	Positive	23.3	28	22.2
7	88/M	11	13	Positive	Positive	22.7	27.4	25.4
8	87/M	NA	14	Negative	Negative	NA	NA	21.2
9	73/M	41	20	Negative	Positive	31.2	33.2	23.8
10	78/M	17	40	Positive	Positive	22.3	26.9	26
11	87/F	4	63	Positive	Positive	16	20.8	25.7
12	70/M	3	65	Positive	Positive	18.2	22.6	22.6
13	84/F	1	57	Positive	Positive	23.8	28.1	27.5
14	90/F	19	41	Negative	Positive	37.3	NA	24.2
15	76/M	27	29	Negative	Negative	NA	NA	23.5
16	78/F	7	25	Positive	Positive	17.3	22.2	21.9
17	76/M	12	14	Positive	Positive	18.9	23.5	25.1
18	62/M	34	18	Negative	Positive	33.9	NA	23.2
19	90/F	4	124	Positive	Positive	24.7	29.2	26.5
20	67/M	12	23	Positive	Positive	26.9	29.7	26.7
21	73/F	10	22	Positive	Positive	22.2	25.7	23.5
22	73/F	12	20	Positive	Positive	21.5	24.4	20.8
23	80/F	12	10	Positive	Positive	22.8	25.9	25.3
24	77/M	43	15	Negative	Negative	NA	NA	25.7
25	93/F	2	16	Positive	Positive	17.5	20.8	23.6
26	87/M	26	29	Negative	Negative	NA	NA	24.2
27	91/M	21	23	Negative	Positive	33.2	NA	23.5
28	77/F	NA	23	Negative	Negative	NA	NA	21
29	79/M	33	17	Negative	Negative	NA	NA	29.7
30	87/M	7	8	Positive	Positive	14.1	18	25.4

*C_t, cycle threshold; E, envelope; GAPDH, human glyceraldehyde 3-phosphate dehydrogenase; N, nucleocapsid; NA, not applicable; RAT, rapid antigen test.
†Interval from first positive (antemortem) SARS-CoV-2 PCR to death.
‡interval from death to specimen sampling.

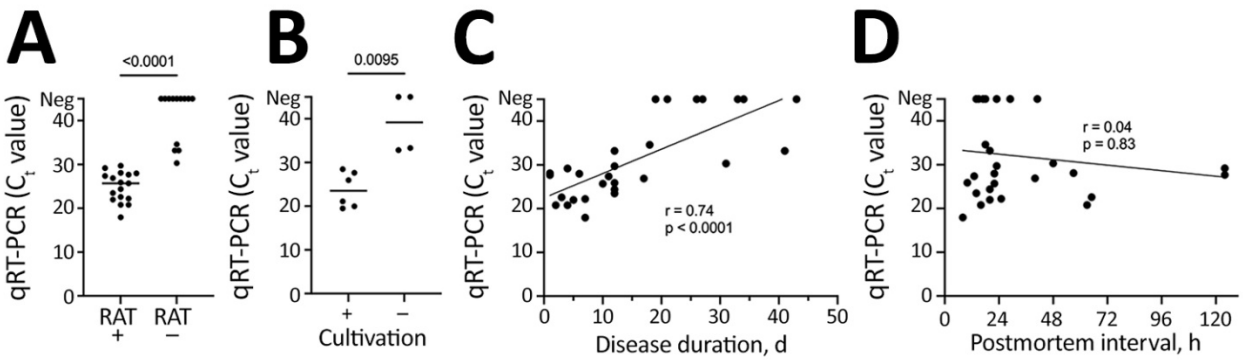
Appendix Table 2. Case characteristics and postmortem data of the cultivation cohort*

Case no.	Age, y/sex	Disease duration, d†	Postmortem interval, h‡	Cultivation	qRT-PCR	C_t (E gene) nasopharynx	C_t (E gene) lung	C_t (N gene) nasopharynx	C_t (N gene) lung
1	78/M	10	28	Positive	Positive	23.7	21.3	29.7	27.7
2	82/M	7	15	Positive	Positive	18.2	17.2	25.3	NP
3	78/M	9	68	Negative	Positive	32.1	32.1	33.7	33.3
4	92/F	3	14	Positive	Positive	17.9	16.6	21.5	20
5	71/F	4	30	Positive	Positive	14.7	14.5	18.4	19.5
6	93/F	9	20	Positive	Positive	13.7	16.5	17.3	21.1
7	79/M	14	25	Negative	Positive	26.3	29.5	30	32.8
8	67/M	20	23	Negative	Positive	36	34.4	NA	NA
9	80/F	7	46	Positive	Positive	18.9	22.5	22.7	26
10	80/F	11	23	Positive	Positive	19.9	25.5	23.9	28.5
11	65/M	34	57	Negative	Positive	30.6	NA	32.4	NA

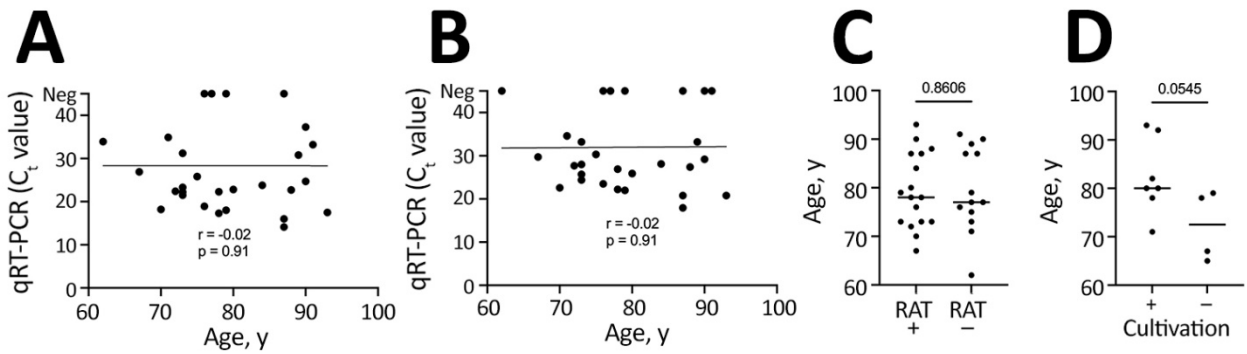
* C_t, cycle threshold; E, envelope; N, nucleocapsid; NA, not applicable; NP, not performed; RAT, rapid antigen test.
†Interval from first positive (antemortem) SARS-CoV-2 PCR to death.
‡Interval from death to specimen sampling.



Appendix Figure 1. E gene qRT-PCR has a higher sensitivity than N gene qRT-PCR. A) More negative cases in N gene qRT-PCR compared with E gene qRT-PCR**.** B) Significantly higher C_t values in N gene qRT-PCR compared with E gene qRT-PCR (Wilcoxon matched-pairs signed rank test). C_t, cycle threshold; E, envelope; N, nucleocapsid; RAT, rapid antigen test.



Appendix Figure 2. N gene qRT-PCR results. A) RAT negative cases show significantly higher C_t values compared with RAT positive cases (Mann-Whitney test). B) Cultivation negative and positive cases mirror C_t values of RAT results (Mann-Whitney test). C) Longer disease durations are significantly correlated with higher C_t values (Spearman correlation test). D) No significant correlation between postmortem intervals and C_t values (Mann-Whitney test). C_t, cycle threshold; E, envelope; N, nucleocapsid; RAT, rapid antigen test.



Appendix Figure 3. qRT-PCR, RAT, and cultivation results in relation to patient age. A–D) No significant correlation between age and C_t values (Spearman correlation test), RAT results (Mann-Whitney test), or cultivation results (Mann-Whitney test). C_t, cycle threshold; E, envelope; N, nucleocapsid; RAT, rapid antigen test.